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(54) METHOD AND COMPOSITIONS FOR THE SYNTHESIS OF BCH-189 AND RELATED COMPOUNDS

VERFAHREN UND ZUSAMMENSETZUNGEN ZUR SYNTHESE VON BCH-189 UND VERWANDTEN VERBINDUNGEN

PROCEDE ET COMPOSITIONS DE SYNTHESE DE BCH-189 ET DE COMPOSES APPARENTES

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Description

TECHNICAL FIELD

The present invention relates to methods and compositions for preparing antiviral nucleoside analogs, particularly BCH-189 (2',3'-dideoxy-3-thiacytosine). More particularly, the invention relates to the selective synthesis of the β -isomer of BCH-189 and related compounds as well as the selective synthesis of enantiomerically-enriched BCH-189 and related compounds.

BACKGROUND ART

In 1981, documentation began on the disease that became known as Acquired Immune Deficiency Syndrome (AIDS), as well as its forerunner AIDS Related Complex (ARC). In 1983, the cause of the disease AIDS was established as a virus named the Human Immunodeficiency Virus type 1 (HIV-1). Usually, a person infected with the virus will eventually develop AIDS; in all known cases of AIDS the final outcome has always been death.

The disease AIDS is the end result of an HIV-1 virus following its own complex life cycle. The virus life cycle begins with the virus attaching itself to the host human T-4 lymphocyte immune cell through the bonding of a glycoprotein on the surface of the virus's protective coat with the CD4 glycoprotein on the lymphocyte cell. Once attached, the virus sheds its glycoprotein coat, penetrates into the membrane of the host cell, and uncoats its RNA. The virus enzyme, reverse transcriptase, directs the process of transcribing the RNA into single stranded DNA. The viral RNA is degraded and a second DNA strand is created. The now double-stranded DNA is integrated into the human cell's genes and those genes are used for cell reproduction.

At this point, the human cell carries out its reproductive process by using its own RNA polymerase to transcribe the integrated DNA into viral RNA. The viral RNA is translated into glycoproteins, structural proteins, and viral enzymes, which assemble with the viral RNA intact. When the host cell finishes the reproductive step, a new virus cell, not a T-4 lymphocyte, buds forth. The number of HIV-1 virus cells thus grows while the number of T-4 lymphocytes decline.

The typical human immune system response, killing the invading virus, is taxed because a large portion of the virus's life cycle is spent in a latent state within the immune cell. In addition, viral reverse transcriptase, the enzyme used in making a new virus cell, is not very specific, and causes transcription mistakes that result in continually changed glycoproteins on the surface of the viral protective coat. This lack of specificity decreases the immune system's effectiveness because antibodies specifically produced against one glycoprotein may be useless against another, hence the virus continues to grow while the immune response reducing the number of antibodies available to fight the virus. The virus continues to grow while the immune response system continues to weaken. Eventually, the HIV largely holds free reign over the body's immune system, allowing opportunistic infections to set in and ensuring that, without the administration of antiviral agents and/or immunomodulators, death will result.

There are three critical points in the virus's life cycle which have been identified as targets for antiviral drugs: (1) the initial attachment of the virus to the T-4 lymphocyte, or macrophage, site, (2) the transcription of viral RNA to viral DNA, and (3) the assembly of the new virus cell during reproduction.

Inhibition of the virus at the second critical point, the viral RNA to viral DNA transcription process, has provided the bulk of the therapies used in treating AIDS. This transcription must occur for the virus to reproduce because the virus's genes are encoded in RNA; the host cell reads only DNA. By introducing drugs that block the reverse transcriptase from completing the formation of viral DNA, HIV-1 replication can be stopped.

Nucleoside analogs, such as 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (DDC), 2',3'-dideoxythymidine (DAT), 2',3'-dideoxyinosine (DDI), and various fluoro-derivatives of these nucleosides are relatively effective in inhibiting HIV replication at the reverse transcriptase stage. Another promising reverse transcriptase inhibitor is 2',3'-dideoxy-3-thiacytosine (BCH-189), which contains an oxathiolane ring substituting for the sugar moiety in the nucleoside.

AZT is a successful anti-HIV drug because it subvertes the formation of viral DNA inside the host T-4 lymphocyte cell. When AZT enters the cell, cellular kinases activate AZT by phosphorylation to AZT triphosphate. AZT triphosphate then competes with natural thymidine nucleosides for the receptor site of HIV reverse transcriptase enzyme. The natural nucleoside possesses two reactive ends, the first for attachment to the previous nucleoside and the second for linking to the next nucleoside. The AZT molecule has only the first reactive end; once inside the HIV enzyme site, the AZT azido group terminates viral DNA formation because the azide cannot make the 3',5'-phosphodiester with the ribose moiety of the following nucleoside.

AZT's clinical benefits include increased longevity, reduced frequency and severity of opportunistic infections, and increased peripheral CD4 lymphocyte count. Immunosorbent assays for viral p24, an antigen used to track HIV-1 activity, show a significant decrease with use of AZT. However, AZT's benefits must be weighed against the severe adverse reactions of bone marrow suppression, nausea, myalgia, insomnia, severe headaches, anemia, peripheral neuropathy, and seizures. Furthermore, these adverse side effects occur immediately after treatment begins whereas a minimum

of six weeks of therapy is necessary to realize AZT's benefits.

Both DDC and DAT are potent inhibitors of HIV replication with activities comparable (DAT) or superior (DDC) to AZT. However, both DDC and DAT are converted to their 5' triphosphates less efficiently than their natural analogs and are resistant to diaminases and phosphatases. Clinically, both compounds are toxic. Currently, DDI is used in combination with AZT to treat AIDS. However, DDI's side effects include aporadic pancreatitis and peripheral neuropathy. Initial tests on 3'-fluoro-2',3'-dideoxythymidine show that its anti-viral activity is comparable to that of AZT.

Recent tests on BCH-189 have shown that it possesses anti-HIV activity similar to AZT and DDC, but without the cell toxicity which causes the debilitating side effects of AZT and DDC. A sufficient quantity of BCH-189 is needed to allow clinical testing and treatment using the drug.

The commonly-used chemical approaches for synthesizing nucleosides or nucleoside analogs can be classified into two broad categories: (1) those which modify intact nucleosides by altering the carbohydrate, the base, or both and (2) those which modify carbohydrates and incorporate the base, or its synthetic precursor, at a suitable stage in the synthesis. Because BCH-189 substitutes a sulfur atom for a carbon atom in the carbohydrate ring, the second approach is feasible. The most important factor in this latter strategy involves delivering the base from the β -face of the carbohydrate ring in the glycosylation reaction because only the β -isomers exhibit useful biological activity.

It is well known in the art that the stereoselective introduction of bases to the anomeric centers of carbohydrates can be controlled by capitalizing on the neighboring group participation of a 2'-substituent on the carbohydrate ring (Chem. Rev. 114:1234 (1981)). However, BCH-189 and its analogs do not possess a 2'-substituent and, therefore, cannot utilize this procedure unless additional steps to introduce a functional group that is both directing and disposable are incorporated into the synthesis. These added steps would lower the overall efficiency of the synthesis.

It is also well known in the art that "considerable amounts of the undesired α -nucleosides are always formed during the synthesis of 2'-deoxyribosides" (Chem. Rev. 114:1234, 1244 (1981)). Furthermore, the reference teaches that the use of simple Friedel-Crafts catalysts like SnCl_4 in nucleoside syntheses produces undesirable emulsions upon the workup of the reaction mixture, generates complex mixtures of the α and β -isomers, and leads to stable α -complexes between the SnCl_4 and the more basic silylated heterocycles such as silylated cytosine. These complexes lead to longer reaction times, lower yields, and production of the undesired unnatural N-S-nucleosides. Thus, the prior art teaches the use of trimethylsilyl triflate or trimethylsilyl perchlorate as a catalyst during the coupling of pyrimidine bases with a carbohydrate ring to achieve high yields of the biologically active β -isomers. However, the use of these catalysts to synthesize BCH-189 or BCH-189 analogs does not produce the β -isomer preferentially; these reactions result in approximately a 50:50 ratio of the isomers.

Thus, there exists a need for an efficient synthetic route to BCH-189 and its analogs. There also exists a need for a stereoselective synthetic route to the biologically active isomer of these compounds, β -BCH-189 and related β -analogues. Furthermore, there exists a need for a stereoselective synthetic route to enantiomerically-enriched β -BCH-189 because the other enantiomer is inactive and, therefore, represents a 50% impurity.

DISCLOSURE OF INVENTION

The present invention relates to the discovery of a surprisingly efficient synthetic route to BCH-189 and various analogs of BCH-189 from inexpensive precursors with the option of introducing functionality as needed. This synthetic route allows the stereoselective preparation of the biologically active isomer of these compounds, β -BCH-189 and enantiomerically-enriched β -BCH-189 and its analogs. Furthermore, the stereochemistry at the nucleoside 4' position can be controlled to produce enantiomerically-enriched β -BCH-189 and its analogs.

The term "BCH-189 analogs" is meant to refer to nucleosides that are formed from pyrimidine bases substituted at the 5 position that are coupled to substituted 1,3-oxathiolanes.

The method of the present invention includes covalently an allyl ether or ester having the formula $\text{CH}_2=\text{CH}-\text{CH}_2-\text{OR}$, in which R is a protecting group, such as an alkyl, allyl, or acyl group, to form a glycoaldehyde having the formula $2-(\text{R-oxymethyl-5-oxo-1,3-oxathiolane})-\text{CHO}$; adding thioglycolic acid to the glycoaldehyde to form a lactone of the formula 2-(R-oxymethyl-5-oxo-1,3-oxathiolane); converting the lactone to its corresponding carboxylate at the 5 position of the oxathiolane ring; coupling the carboxylate with a silylated pyrimidine base in the presence of SnCl_4 to form the β -isomer of a 5-(R-oxymethyl-2',3'-dideoxy-5-thia- nucleoside analog; and replacing the R protecting group with a hydrogen to form BCH-189 or an analog of BCH-189.

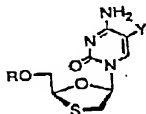
The invention can be used to produce BCH-189 or BCH-189 analogs that are enantiomerically-enriched at the 4' position by selecting an appropriate R protecting group to allow stereoselective selection by an enzyme. For instance, position by selecting an appropriate R protecting group to allow stereoselective selection by an enzyme. For instance, the R protecting group can be chosen such that the substituent at the 2 position of the oxathiolane lactone is butyryloxy to permit stereoselective enzymatic hydrolysis by pig liver esterase. The resulting optically active hydrolyzed lactone can then be converted to its corresponding diacetate and coupled with a silylated pyrimidine base as above.

Accordingly, one of the objectives of this invention is to provide an efficient method for preparing the β -isomer of BCH-189 and analogs of BCH-189 in high yields. Furthermore, it is an objective of this invention to provide a synthetic

method to produce only one optical isomer, rather than a racemic mixture, of BCH-189 and analogs of BCH-189. A further object of this invention is to provide a synthetic route to produce R-BCH-189 that is enantiomerically-enriched.

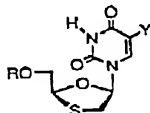
Additionally, an objective of this invention is to provide intermediates from which BCH-189 or BCH-189 analogs can be synthesized of the formula 2-(R-oxymethyl)-5-acyloxy-1,3-oxathiolane, wherein R is a protecting group, such as alkyl, silyl, or acyl, and a method of preparing these compounds. Furthermore, it is an object of this invention to provide enantiomerically-enriched 2-acetoxymethyl-5-acetoxy-1,3-oxathiolane and 2-butoxymethyl-5-oxo-1,3-oxathiolane and methods of preparing these compounds.

Another objective of this invention is to provide intermediates from which BCH-189 or BCH-189 analogs can be synthesized of the formula:



wherein R is a protecting group, such as alkyl, silyl, or acyl, and Y can be hydrogen, methyl, halo, alkyl, alkenyl, alkynyl, hydroalkyl, carbalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl, and methods of preparing these compounds.

Furthermore, this invention provides intermediates from which BCH-189 or BCH-189 analogs can be synthesized of the formula:



wherein R is a protecting group, such as alkyl, silyl, or acyl, and Y can be hydrogen, methyl, halo, alkyl, alkenyl, alkynyl, hydroalkyl, carbalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl, and methods of preparing these compounds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates one embodiment of a synthesis of BCH-189 and BCH-189 analogs according to the present invention;

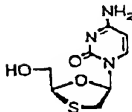
Figure 2 illustrates one embodiment of the synthesis of BCH-189 according to the present invention;

Figure 3 illustrates one embodiment of the synthesis of 5-methylcytidine and thymidine derivatives of BCH-189 according to the present invention; and

Figure 4 illustrates one embodiment of the synthesis of enantiomerically-enriched BCH-189 according to the present invention.

BEST MODE OF CARRYING OUT THE INVENTION

BCH-189 is a compound of the formula:



5

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The process of the present invention for preparing BCH-189 and BCH-189 analogs is set forth in Fig. 1. An allyl ether or ester 1 is ozonized to give an aldehyde 2, which reacts with triglycolic acid to give a lactone 3. The lactone 3 is treated with a reducing agent, followed by a carboxylic anhydride, to produce the carboxylate 4. This carboxylate is coupled with a silylated pyrimidine base in the presence of a Lewis acid that can catalyze stereospecific coupling, such as SnCl_4 , to yield the β -isomer of the substituted nucleoside 5 in essentially a 100:0 ratio of β : α isomers. The substituted nucleoside 5 is deprotected to produce BCH-189 or BCH-189 analog 6.

This procedure can be tailored to produce BCH-189 or BCH-189 analogs that are enantiomerically-enriched at the 4' position by selecting an appropriate R protecting group to allow stereoselective enzymatic hydrolysis of 2 by an enzyme such as pig liver esterase, porcine pancreatic lipase, or subtilisin or other enzymes that hydrolyze 3 in a stereoselective fashion. The resulting optically active 2 can be converted to enantiomerically-enriched carboxylate 4 and coupled with a silylated pyrimidine base as above to produce enantiomerically-enriched BCH-189 or BCH-189 analogs.

The protecting group R in 1 can be selected to provide protection for the corresponding alcohol until the final step in the synthesis is carried out (deprotection of 5 to form 6). Additionally, the protecting group can be selected, if desired, to provide an additional recognition site for an enzyme to be used later in an enantio-selective hydrolysis reaction. Any group that functions in this manner may be used. For instance, alkyl, silyl, and acyl protecting groups or groups that possess substantially the same properties as these groups can be used.

An alkyl protecting group, as used herein, means triphenylmethyl or an alkyl group that possesses substantially the same protecting properties as triphenylmethyl. A silyl protecting group, as used herein, means a trialkylsilyl group having the formula:



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wherein R_1 , R_2 , and R_3 may be lower-alkyl, e.g., methyl, ethyl, butyl, and alkyl possessing 5 carbon atoms or less; or phenyl. Furthermore, R_1 may be identical to R_2 ; R_1 , R_2 , and R_3 may all be identical. Examples of silyl protecting groups include, but are not limited to, trimethylsilyl and t-butylphenylsilyl.

An acyl group, as used herein to describe an acyl protecting group (as in 1) or to describe a carboxylate (as in 4), is a group having the formula:



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wherein R is a lower alkyl, e.g., methyl, ethyl, butyl, and alkyl possessing 5 carbon atoms or less; substituted lower alkyl wherein the alkyl bears one, two, or more simple substituents, including, but not limited to, amino, carbonyl, hydroxy, phenyl, lower-alkoxy, e.g., methoxy and ethoxy; phenyl; substituted phenyl wherein the phenyl bears one, two, or more simple substituents, including, but not limited to, lower alkyl, halo, e.g., chloro and bromo, sulfo, sulfonyloxy, carboxyl, carbo-lower-alkoxy, e.g., carbomethoxy and carbethoxy, amino, mono- and di-lower alkylamino, e.g., methylamino,

amido, hydroxy, lower alkoxy, e.g., methoxy and ethoxy, lower-alkanoyloxy, e.g., acetoxy.
A silylated pyrimidine base, as used herein, means a compound having the formula:



wherein X is either a trialkylsilyloxy or a trialkylsilylamino group, Z is a trialkylsilyl group, and Y is further described below. A trialkylsilyl group, as used herein, means a group having the formula:



wherein R₁, R₂, and R₃ may be lower-alkyl, e.g., methyl, ethyl, butyl, and alkyl possessing 5 carbon atoms or less, or phenyl. Furthermore, R₁ may be identical to R₂, R₁, R₂, and R₃ may all be identical. Examples of trialkylsilyl groups include, but are not limited to, trimethylsilyl and t-butyl-diphenylsilyl.

The silylated pyrimidine base may be substituted with various Y substituents, including, but not limited to, hydrogen, methyl, halo, alkyl, allyl, alkenyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenoxy, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl, at position 6 of the silylated pyrimidine base (Y substituent in Fig. 1) to modify the properties, such as transport properties or the rate of metabolism, of the BCH-189 analog.

Illustrative examples of the synthesis of BCH-189 or BCH-189 analogs according to the present invention are given in Figs. 2, 9, and 4 and the following descriptions.

In Figs. 2, 9, and 4 and the following descriptions, Figure 2 shows the synthesis of BCH-189 starting with allyl alcohol Z. A NaH oil suspension (4.5 g, 60%, 110 mmol) was washed with THF twice (100 ml x 2) and the resulting solid suspended in THF (200 ml). The suspension was cooled to 0°C, allyl alcohol Z (5.8 ml, 100 mmol) was added dropwise, and the mixture was stirred for 30 minutes at 0°C. t-Butyl-diphenylsilyl chloride (25.6 ml, 100.8 mmol) was added dropwise at 0°C and the reaction mixture was stirred for 1 hour at 0°C. The solution was quenched with water (100 ml), and extracted with diethyl ether (200 ml x 2). The combined extracts were washed with water, dried over MgSO₄, filtered, concentrated, and the residue distilled under vacuum (90-100°C at 0.5-0.6 mm Hg) to give a colorless liquid 8 (28 g, 84 mmol, 84%). ¹H NMR: 7.70-7.85 (10H, m, aromatic-H); 5.93 (1H, m, H₂); 5.37 (1H, dt, H₁) J=1.4 and 14.4 Hz; 5.07 (1H, dt, H₁) J=1.4 and 8.7 Hz; 4.21 (2H, m, H₂); 1.07 (9H, s, t-Bu)

The silyl allyl ether 8 (15.5 g, 52.3 mmol) was dissolved in CH₂Cl₂ (400 ml), and ozonized at -78°C. Upon completion of ozonolysis, DMS (15 ml, 204 mmol, 5.9 eq) was added at -78°C and the mixture was warmed to room temperature and stirred overnight. The solution was washed with water (100 ml x 2), dried over MgSO₄, filtered, concentrated, and distilled under vacuum (100-110°C at 0.5-0.6 mm Hg) to give a colorless liquid 9 (15.0 g, 50.3 mmol, 95%). ¹H NMR: 9.74 (1H, s, H-CO); 7.70-7.35 (10H, m, aromatic-H); 4.21 (2H, s, -CH₂); 1.22 (9H, s, t-Bu)

Silylated glyoxaldehyde 9 (15.0 g, 50.3 mmol) was dissolved in toluene (200 ml) and thioglycolic acid (3.50 ml, 50.3 mmol) was added all at once. The solution was refluxed for 2 hours while the resulting water was removed with a Dean-Stark trap. The solution was cooled to room temperature and washed with saturated NaHCO₃ solution and water (100 ml x 2), washed again with diethyl ether (200 ml x 2). The combined extracts were washed with water (100 ml x 2), dried over MgSO₄, filtered, and concentrated to give a colorless oil 10 (16.5 g, 44.3 mmol, 88%), which gradually solidified over MgSO₄. Recrystallization from hexane afforded a white solid 10 (15.2 g, 84%). ¹H NMR: 7.72-7.98 (10H, m, aromatic-H); 5.63 (1H, t, H₂) J=2.7 Hz; 3.93 (1H, dt, -CH₂O) J=5.3 Hz; 3.81 (1H, d, 1H₂) J=13.8 Hz; 9.79 (1H, dd, -CH₂O); 3.58 (1H, d, 1H₂); 1.02 (9H, s, t-Bu)

2-(t-Butyl-diphenylsilyloxy)-methyl-5-oxo-1,2-oxathiolane 10 (5.0 g, 13.42 mmol) was dissolved in toluene (150 ml) and the solution was cooled to -78°C. Dibal-H solution (14 ml, 1.0 M in hexanes, 14 mmol) was added dropwise, while the inside temperature was kept below -70°C all the time. After the completion of the addition, the mixture was stirred for 30 minutes at -78°C. Acetic anhydride (5 ml, 53 mmol) was added and the mixture was warmed to room temperature and stirred overnight. Water (5 ml) was added to the mixture and the resulting mixture was stirred for 1 hour at room temperature. The mixture was diluted with diethyl ether (300 ml), MgSO₄ (40 g) was added, and the mixture was stirred

Vigorously for 1 hour at room temperature. The mixture was filtered, concentrated, and the residue flash chromatographed with 20% EtOAc in hexanes to give a colorless liquid **11** (3.60 g, 8.64 mmol, 64%), which was a 6:1 mixture of isomers. (¹H NMR of the major isomer: 7.70-7.35 (10H, m, aromatic-H); 6.63 (1H, d, H₃) J=4.4 Hz; 5.47 (1H, t, H₂); 4.20-3.60 (2H, m, -CH₂O-); 3.27 (1H, dd, 1H₄) J=4.4 and 11.4 Hz; 3.09 (1H, d, 1H₄) J=11.4 Hz; 2.02 (3H, s, CH₃CO-); 1.05 (9H, s, t-Bu)). ¹H NMR of the minor isomer: 7.70-7.35 (10H, m, aromatic-H); 6.55 (1H, d, H₃) J=3.9 Hz; 5.45 (1H, t, H₂); 4.20-3.60 (2H, m, -CH₂O-); 3.26 (1H, dd, 1H₄) J=3.9 and 11.4 Hz; 3.11 (1H, d, 1H₄) J=11.4 Hz; 2.04 (3H, s, CH₃CO-); 1.04 (9H, s, t-Bu)).

2-(1-Butyl-diphenylsilyl)-methyl-5-acetoxyl-1,3-oxathiolane **11** (0.28 g, 0.67 mmol) was dissolved in 1,2-dichloroethane (20 ml), and silylated cytosine **12** (0.20 g, 0.78 mmol) was added at once at room temperature. The mixture was stirred for 10 minutes and to it was added SnCl₄ solution (0.80 ml, 1.0 M solution in CH₂Cl₂, 0.80 mmol) dropwise at room temperature. Additional cytosine **12** (0.10 g, 0.39 mmol) and SnCl₄ solution (0.60 ml) were added in a same manner 1 hour later. After completion of the reaction in 2 hours, the solution was concentrated, and the residue was treated with triethylamine (2 ml) and subjected to flash chromatography (first with neat EtOAc and then 20% ethanol in EtOAc) to give a tan solid **13** (100% β configuration) (0.25 g, 0.54 mmol, 80%) (¹H NMR (DMSO-d₆): 7.75 (1H, d, H₃) J=7.5 Hz; 7.55-7.25 (10H, m, aromatic-H); 7.21 and 7.14 (2H, broad, -NH₂); 6.19 (1H, t, H₂); 5.57 (1H, d, H₂); 5.25 (1H, d, H₂); 3.97 (1H, dd, -CH₂O-); 3.5 and 11.1 Hz; 3.87 (1H, dd, -CH₂O-); 3.41 (1H, dd, 1H₄) J=4.5 and 11.7 Hz; 3.03 (1H, dd, 1H₄) J=7.97 (9H, s, t-Bu)).

Silyl ether **13** (0.23 g, 0.49 mmol) was dissolved in THF (30 ml), and to it was added n-Bu₄NF solution (0.50 ml, 1.0 M solution in THF, 0.50 mmol) dropwise at room temperature. The mixture was stirred for 1 hour and concentrated under vacuum. The residue was taken up with ethanol/triethylamine (2 ml/1 ml), and subjected to flash chromatography (first with EtOAc, then 20% ethanol in EtOAc) to afford a white solid **14** in 100% anomeric purity (BCH-189; 0.11 g, 0.48 mmol, 96%), which was further recrystallized from ethanol/CHCl₃/Hexanes mixture. (¹H NMR (DMSO-d₆): 7.91 (1H, d, H₃) J=7.6 Hz; 7.78 and 7.45 (2H, broad, -NH₂); 6.19 (1H, t, H₂); 5.80 (1H, d, H₂) J=7.6 Hz; 5.34 (1H, broad, -OH); 5.17 (1H, t, H₂); 3.74 (2H, m, -CH₂O-); 3.42 (1H, dd, 1H₄) J=5.6 and 11.5 Hz; 3.09 (1H, dd, 1H₄) J=4.5 and 11.5 Hz).

BCH-189 and its analogs can also be synthesized by coupling a silylated uracil derivative with **11**. Silylated uracil derivative **15** (1.80 g, 7.02 mmol) was coupled with **11** (1.72 g, 4.18 mmol) in 1,2-dichloroethane (30 ml) in the presence of SnCl₄ (5.0 ml) as described above in the preparation of the cytosine derivative **13**. The reaction was complete after 5 hours. Flash chromatography, first with 40% EtOAc in hexanes and then EtOAc, afforded a white solid **16** (1.60 g, 3.43 mmol, 83%). (¹H NMR: 9.39 (1H, broad, -NH); 7.90 (1H, d, H₃) J=7.9 Hz; 7.75-7.35 (10H, m, aromatic-H); 6.33 (1H, dd, H₂); 5.51 (1H, d, H₂) J=7.9 Hz; 5.23 (1H, t, H₂); 4.11 (1H, dd, -CH₂O-); 3.2 and 11.7 Hz; 3.93 (1H, dd, -CH₂O-); 3.48 (1H, dd, 1H₄) J=5.4 and 12.2 Hz; 3.18 (1H, dd, 1H₄) J=3.2 and 12.2 Hz).

The uracil derivative **16** can be converted to the cytosine derivative **18**. The uracil derivative **16** (0.20 g, 0.43 mmol) was dissolved in a mixture of pyridine/dichloroethane (2 ml/10 ml), and the solution cooled to 0°C. Triisic anhydride (72 μl, 0.43 mmol) was added dropwise at 0°C and the mixture was warmed to room temperature and stirred for 1 hour. Additional triisic anhydride (0.50 μl, 0.30 mmol) was added and the mixture stirred for 1 hour. TLC showed no mobility with EtOAc. The reaction mixture was then decannulated into a NH₂-saturated methanol solution (30 ml) and the mixture was stirred for 12 hours at room temperature. The solution was concentrated, and the residue subjected to flash chromatography to give a tan solid **18** (0.18 g, 0.39 mmol, 91%), which was identical with the compound obtained from the cytosine coupling reaction.

Fig. 3 illustrates the synthesis of 5-methylcytosine and thymidine derivatives of BCH-189. The acetate **11** (0.93 g, 2.23 mmol) in 1,2-dichloroethane (50 ml) was reacted with the silylated thymine derivative **12** (1.0 g, 3.70 mmol) and SnCl₄ solution (4.0 ml) in a manner similar to that described for the preparation of cytosine derivative **13**. (¹H NMR: 8.10 (1H, broad, -NH); 7.75-7.80 (11H, m, 10 Aromatic H's and 1H₃); 6.52 (1H, t, H₂) J=5.4 Hz; 5.25 (1H, t, H₂) J=4.2 Hz; 4.01 (1H, dd, 1H₄) J=3.8 and 11.4 Hz; 3.93 (1H, dd, 1H₄) J=4.5 and 11.4 Hz; 3.52 (1H, d, 1H₄) J=5.4 and 11.7 Hz; 3.04 (1H, dd, 1H₄) J=5.7 and 11.7 Hz; 1.07 (9H, s, CH₃); 1.07 (9H, s, t-Bu)).

The thymine derivative **18** (0.20 g, 0.42 mmol) was dissolved in a mixture of pyridine/dichloroethane (2 ml/10 ml), and the solution cooled to 0°C. To it was added triisic anhydride (100 μl, 0.60 mmol) dropwise at 0°C, and the mixture was allowed, with continuous stirring, to warm to room temperature. After reaching room temperature, it was stirred for 1 hour. TLC showed no mobility with EtOAc. The reaction mixture was then decannulated into the NH₂-saturated methanol solution (20 ml), and the mixture stirred for 12 hours at room temperature. The solution was concentrated, and the residue was subjected to flash chromatography to give a tan solid **19** (0.18 g, 0.38 mmol, 90%). (¹H NMR: 7.70-7.30 (12H, m, 10 Aromatic H's, 1NH and H₃); 6.50 (1H, broad, -NH); 6.34 (1H, t, H₂) J=4.5 Hz; 5.25 (1H, t, H₂) J=3.5 Hz; 4.08 (1H, dd, 1H₄) J=3.6 and 11.4 Hz; 3.96 (1H, dd, 1H₄) J=3.6 and 11.4 Hz; 3.52 (1H, d, 1H₄) J=5.4 and 12.3 Hz; 3.09 (1H, dd, 1H₄) J=3.9 and 12.3 Hz; 1.72 (3H, s, CH₃); 1.07 (9H, s, t-Bu)).

Silyl ether **19** (0.18 g, 0.38 mmol) was dissolved in THF (20 ml), and n-Bu₄NF solution (0.50 ml, 1.0 M solution in THF, 0.50 mmol) was added, dropwise, at room temperature. The mixture was stirred for 1 hour and concentrated under vacuum. The residue was taken up with ethanol/triethylamine (2 ml/1 ml), and subjected to flash chromatography (first with EtOAc, then 20% ethanol in EtOAc) to afford a white solid **20** (0.09 g, 0.37 mmol, 97%), which was further

recrystallized from ethanol/CH₂Cl₂/hexanes mixture to afford 82 mg of pure compound (89%). (¹H NMR: (in d⁶-DMSO): 7.70 (1H, s, H₆); 7.48 and 7.10 (2H, broad, NH₂); 6.19 (1H, t, H₃) J=5.5 Hz; 5.81 (1H, t, OH); 5.16 (1H, t, H_{4a}) J=5.4 Hz; 3.72 (2H, m, 2H₅); 3.38 (1H, dd, 1H₂) J=6.5 and 14.0 Hz; 3.05 (1H, dd, 1H₂) J=8.5 and 14.0 Hz; 1.85 (3H, s, CH₃).

Silyl ether 18 (0.70 g, 4.46 mmol) was dissolved in THF (50 ml), and an n-Bu₄NF solution (2 ml, 1.0 M solution in THF, 2 mmol) was added, dropwise, at room temperature. The mixture was stirred for 1 hour and concentrated under vacuum. The residue was taken up with ethanol/triethylamine (2 ml/1 ml), and subjected to flash chromatography to afford a white solid 21 (0.33 g, 1.35 mmol, 92%). (¹H NMR: (in d⁶-Acetone): 9.98 (1H, broad, NH₂); 7.78 (1H, d, H₆) J=1.2 Hz; 6.25 (1H, t, H₄) J=5.7 Hz; 6.24 (1H, t, H₃) J=4.2 Hz; 4.39 (1H, t, OH) J=5.7 Hz; 3.85 (1H, dd, 2H₅) J=4.2 and 5.7 Hz; 3.41 (1H, dd, 1H₂) J=5.7 and 12.0 Hz; 3.19 (1H, dd, 1H₂) J=5.4 and 12.0 Hz; 1.80 (3H, s, CH₃).

Fig. 4 illustrates the synthesis of enantiomerically-enriched BCH-189 and its analogs. Allyl butyrate 22 (19.0 g, 148 mmol) was dissolved in CH₂Cl₂ (400 ml), and ozonized at -78°C. Upon completion of ozonolysis, dimethyl sulfide (20 ml, 270 mmol, 1.8 eq) was added at -78°C and the mixture was warmed to room temperature and stirred overnight. The solution was washed with water (100 ml x 2), dried over MgSO₄, filtered, concentrated, and distilled under vacuum (70-80°C at 0.5-0.6 mm Hg) to give a colorless liquid 23 (17.0 g, 131 mmol, 89%). (¹H NMR: 9.59 (1H, s, H-CO); 4.06 (2H, t, s, -CH₂O); 2.42 (2H, t, CH₂CO) J=7.2 Hz; 1.71 (2H, sex, -CH₂); 0.97 (3H, t, CH₃) J=7.2 Hz) (IR (neat): 2960, 2960, 2900, 1750, 1740, 1480, 1420, 1390, 1280, 1190, 1110, 1060, 1020, 990, 880, 800, 760).

Butyrylacyl chloride 23 (15.0 g, 115 mmol) was dissolved in toluene (200 ml) and mixed with thiolglycolic acid (8.0 ml, 115 mmol). The solution was refluxed for 5 hours while the resulting water was removed with a Dean-Stark trap. The solution was cooled to room temperature and was transferred to a 500 ml separatory funnel. The solution was then washed with saturated NaHCO₃ solution. These aqueous washings were extracted with diethyl ether (200 ml x 2) to recoverate any crude product from the aqueous layer. The ether extracts were added to the toluene layer and the resulting mixture was washed with water (100 ml x 2), dried over MgSO₄, filtered, concentrated, and distilled under vacuum (70-80°C at 0.5-0.6 mm Hg) to give a colorless oil 24 (19 g, 93 mmol, 81%). (¹H NMR: 5.85 (1H, dd, H₃) J=5.0 and 1.4 Hz; 4.35 (1H, dd, -CH₂O) J=3.2 and 12.2 Hz; 4.29 (1H, dd, -CH₂O) J=5.7 and 12.2 Hz; 3.72 (1H, d, -CH₂S) J=16.2 Hz; 3.64 (1H, d, -CH₂S); 2.34 (2H, t, -CH₂CO) J=7.2 Hz; 1.66 (2H, sex, -CH₂); 0.95 (3H, t, CH₃) J=7.2 Hz) (IR (neat): 2980, 2960, 2900, 1760, 1740, 1460, 1410, 1390, 1350, 1300, 1290, 1260, 1220, 1170, 1110, 1080, 1070, 1000, 950, 910, 800, 820, 800, 760).

Pig liver esterase solution (80 µl) was added to a buffer solution (pH 7, 100 ml) at room temperature, and the mixture stirred vigorously for 5 minutes. The butyrate 24 (2.8 g, 13.7 mmol) was added, all at once, to the esterase/buffer solution and the mixture was stirred vigorously at room temperature for 2 hours. The reaction mixture was poured into a separatory funnel. The reaction flask was washed with ether (10 ml) and the washing was combined with the reaction mixture in the funnel. The combined mixture was extracted with hexanes three times (100 ml x 3). The three hexane extracts were combined and dried over MgSO₄, filtered, and concentrated to give the optically active butyrate 24 (1.12 g, 5.48 mmol, 40%). Enantiomeric excess was determined by an NMR experiment using a Tris(3-heptafluoropropyl)-hydroxymethylene-(+)-camphorato europium (III) derivative as a chemical shift reagent; this procedure showed approximately 40% enrichment for one enantiomer. The remaining aqueous layer from the reaction was subjected to a continuous extraction with CH₂Cl₂ for 20 hours. The organic layer was removed from the extraction apparatus, dried over MgSO₄, filtered, and concentrated to give an oil (1.24 g), which was shown by NMR analysis to consist of predominantly the 2-hydroxymethyl-5-oxo-1,3-coxathiolane 25 with small amounts of butyric acid and the butyrate 24.

The lactone 25 (0.85 g, 4.16 mmol) was dissolved in toluene (30 ml), and the solution cooled to -78°C. Dibal-H solution (9 ml, 1.0 M in hexanes, 5 mmol) was added dropwise, while the inside temperature was kept below -70°C throughout the addition. After the addition was completed, the mixture was stirred for 0.5 hours at -78°C. Acetic anhydride (5 ml, 53 mmol) was added and the mixture, with continuous stirring, was allowed to reach room temperature overnight. Water (5 ml) was added to the reaction mixture and the resultant mixture was stirred for 1 hour. MgSO₄ (40 g) was then added and the mixture was stirred vigorously for 1 hour at room temperature. The mixture was filtered, concentrated, and the residue flash chromatographed with 20% EtOAc in hexanes to give a colorless liquid 25 (0.41 g, 1.86 mmol, 45%) which was a mixture of anomers at the C-4 position.

The 2-Acetoxyethyl-5-acetoxy-1,3-coxathiolane 26 (0.40 g, 1.82 mmol) was dissolved in 1,2-dichloroethane (40 ml), and to it the silylated cytosine 12 (0.70 g, 2.74 mmol) was added, all at once, at room temperature. The mixture was stirred for 10 minutes, and to it a SnCl₄ solution (3.0 ml, 1.0 M solution in CH₂Cl₂, 3.0 mmol) was added, dropwise, at room temperature. Additional SnCl₄ solution (1.0 ml) was added after 1 hour. The reaction was followed by TLC. Upon completion of the coupling, the solution was concentrated, the residue was triturated with triethylamine (2 ml) and subjected to flash chromatography (first with neat EtOAc then 20% ethanol in EtOAc) to give a tan solid 27 (0.42 g, 1.66 mmol, 86%). (¹H NMR: 7.73 (1H, d, H₆) J=7.5 Hz; 6.33 (1H, t, H_{4a}) J=4.0 Hz; 5.80 (1H, d, H₃) J=5.2 Hz; 4.52 (1H, dd, 1H₂) J=5.7 and 12.3 Hz; 4.37 (1H, dd, 1H₂) J=3.3 and 12.3 Hz; 3.54 (1H, dd, H₂) J=5.4 and 12.0 Hz; 3.10 (1H, dd, 1H₂); 2.11 (3H, s, CH₃)).

The 5'-Acetate of BCH-189 27 (140 mg, 0.52 mmol) was dissolved in anhydrous methanol (10 ml), and to it was added sodium methoxide (110 mg, 2.0 mmol) in one portion. The mixture was stirred at room temperature until the

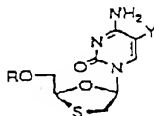
hydrolysis was complete. The hydrolysis took about 1 hour, and the reaction was followed by TLC. Upon completion, the mixture was then concentrated, and the residue taken up with ethanol (2 ml). The ethanol solution was subjected to column chromatography using ethyl acetate first, then 20% ethanol in EtOAc to afford a white foam (110 mg, 92%), which exhibited an NMR spectrum identical to that of authentic BCH-189, 14.

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Claims

1. A method of preparing a nucleoside composition comprising predominantly the β -isomer of the nucleoside 2',3'-dideoxy-3-thiapyrimidine having the formula:

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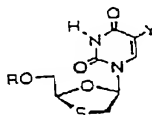


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or

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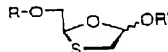
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wherein R is selected from the group consisting of hydrogen, alkyl, silyl, and acyl; and wherein Y is selected from the group consisting of hydrogen, methyl, chloro, fluoro, iodo, bromo, alkyl, silyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl.

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comprising the step of coupling a 1,3-oxathiolane of the structure:

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wherein R' is an acyl group and R is an oxygen-protecting group, with a silylated pyrimidine base in the presence of SnCl_4 , wherein the silylated pyrimidine base has the formula:

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wherein X is selected from the group consisting of tri-alkyl silyloxy and tri-alkyl silylamino; wherein Y is selected from the group consisting of hydrogen, methyl, chloro, fluoro, iodo, bromo, alkyl, silyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and

selenoaryl;

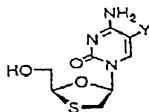
wherein Z is a tri-substituted silyl group having the formula:



wherein R_1 , R_2 , and R_3 are independently selected from alkyl possessing 5 carbon atoms or less and phenyl.

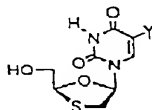
2. The method of claim 1, wherein [R], the oxygen-protecting group, is selected from the group consisting of alkyl, silyl, and acyl.

3. The method of Claim 1, wherein the nucleoside has the formula:



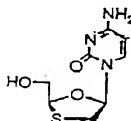
wherein Y is selected from the group consisting of hydrogen, halo, alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl.

4. The method of Claim 1, wherein the nucleoside has the formula:

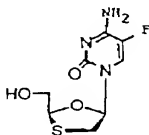


wherein Y is selected from the group consisting of hydrogen, halo, alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl.

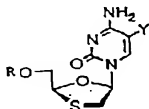
5. The method of Claim 1, wherein the nucleoside has the formula:



6. The method of Claim 1, wherein the nucleoside has the formula:

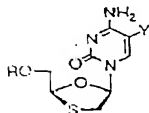


7. The method of Claim 1, further comprising the step of reacting a glycoside having the formula OHCH_2OR , wherein R is an oxygen protecting group with thioglycolic acid to produce a 5-oxo-1,3-oxathiolane.
8. The method of Claim 8 further comprising:
- reducing the carbonyl group in 5-oxo-1,3-oxathiolane to an alcohol; and
 - acylating the alcohol.
9. The method of Claim 8, wherein the reducing agent is selected from the group consisting of diisobutylaluminum hydride, sodium bis(2-methoxyethoxy)aluminum hydride, and NaBH_4 .
10. The method of Claim 1, further comprising replacing R with hydrogen.
11. The method of Claim 10, wherein R is an acyl group and wherein the acyl group is removed with sodium methoxide.
12. Enantiomerically-enriched 2-hydroxymethyl-5-oxo-1,3-oxathiolane.
13. Enantiomerically-enriched 2-acyloxymethyl-5-acyloxy-1,3-oxathiolane.
14. Enantiomerically-enriched 2-acetoxymethyl-5-acetoxy-1,3-oxathiolane.
15. A nucleoside having the formula:



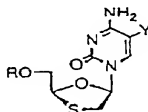
wherein R is selected from the group consisting of hydrogen, alkyl, silyl, and acyl, and
wherein Y is selected from the group consisting of chloro, bromo, fluoro, and iodo.

16. An enantiomerically-enriched nucleoside having the formula:



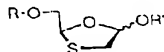
wherein R is selected from the group consisting of hydrogen, alkyl, silyl, and acyl; and wherein Y is selected from the group consisting of hydrogen and fluorine.

17. An enantiomerically-enriched nucleoside having the formula:



wherein R is selected from the group consisting of alkyl, silyl, and acyl; and wherein Y is selected from the group consisting of chloro, bromo, fluoro, and iodo.

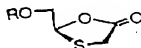
18. A method of preparing a 1,3-oxathiolane having the formula:



wherein R is an oxygen protecting group; and wherein R' is an acyl group, comprising the steps of

(a) oxidizing $\text{CH}_2\text{CH}(\text{OH})\text{OR}$ to form a glycoaldehyde having the formula OHCCH_2OR , wherein R is a protecting group;

(b) adding thio glycolic acid to the glycoaldehyde to form a lactone having the formula: and



(c) reducing the lactone to form the 1,3-oxathiolane

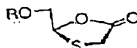
19. The method of Claim 18, wherein the reduction of the lactone is accomplished by an addition of reducing agent followed by addition of a carboxylic anhydride.

20. The method of Claim 18, wherein said reducing agent is selected from the group consisting of diisobutylaluminum hydride, sodium bis(2-methoxyethoxy)aluminum hydride, and NaBH_4 .

21. The method of Claim 18, wherein said protecting group is selected from the group consisting of alkyl, silyl, and acyl.

22. A method of preparing enantiomerically-enriched 2-acyloxymethyl-5-acyloxy-1,3-oxathiolane comprising the steps of:

(a) mixing a stereoselective enzyme with a lactone of the formula



wherein R is an oxygen protecting group to form enantiomerically-enriched 2-hydroxymethyl-5-oxo-1,3-oxathiolane; and

(b) reducing said enantiomerically-enriched 2-hydroxymethyl-5-oxo-1,3-oxathiolane to form enantiomerically-enriched 2-acyloxymethyl-5-acyloxy-1,3-oxathiolane.

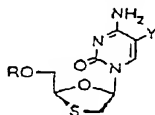
23. The method of Claim 22, wherein the reduction of the enantiomerically-enriched 2-hydroxymethyl-5-oxo-1,3-oxathiolane is accomplished by an addition of a reducing agent followed by addition of a carboxylic anhydride.

24. The method of Claim 22, wherein said reducing agent is selected from the group consisting of diisobutylaluminum hydride, sodium bis(2-methoxyethoxy)aluminum hydride, and NaBH_4 .

25. The method of Claim 22, wherein said stereoselective enzyme is selected from the group consisting of pig liver esterase, porcine pancreatic lipase, and subtilisin.

26. The method of Claim 22, wherein said protecting group is selected from the group consisting of alkyl, silyl, and acyl.

27. A method for resolving the enantiomers of a nucleoside of the structure:



wherein Y is hydrogen or fluorine and R is an acyl group, comprising treating the nucleoside with an enzyme selected from the group consisting of pig liver esterase, porcine pancreatic lipase, and subtilisin.

28. The method of Claim 27, wherein Y is hydrogen.

29. The method of Claim 27, wherein Y is fluorine.

30. The method of Claim 28 or 29, wherein the enzyme is pig liver esterase.

31. The method of Claim 28 or 29, wherein the enzyme is porcine pancreatic lipase.

32. Enantiomerically enriched β -BCH-189.

33. A compound obtainable by the method of Claim 28.

34. A compound obtainable by the method of Claim 29.

35. The compound of Claim 33 which is the (-)-enantiomer.

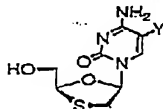
36. The compound of Claim 33 which is the (+)-enantiomer.

37. β -BCH-189 substantially in the form of a single optical isomer.

38. β -BCH-189 as in Claim 37 wherein the isomer is the (+)-enantiomer.

39. β -BCH-189 as in Claim 37 wherein the isomer is the (-)-enantiomer.

40. A compound of the formula:

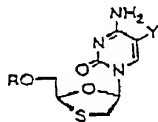


wherein Y is fluoro.

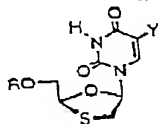
41. The enantiomerically enriched 5-F-cytosine analogue of BCH-189.

Patentanprüche

1. Verfahren zur Herstellung einer Nucleosidmasse, welche vornehmlich das β -Isomer des Nucleosids 2',3'-Dideoxy-5'-thiapyrimidin der Formel:



oder

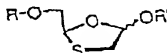


worin bedeuten:

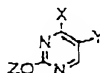
R Wasserstoff, Alkyl, Silyl oder Acyl, und

Y Wasserstoff, Methyl, Chlor, Fluor, Iod, Brom, Alkyl, Alkenyl, Alkynyl, Hydroxyalkyl, Carboxyalkyl, Thioalkyl, Selenoalkyl, Phenyl, Cycloalkyl, Cycloalkenyl, Thioaryl oder Selenoaryl

umfaßt, durch:



worin R' für eine Acylgruppe steht und R eine Sauerstoffschutzgruppe darstellt, mit einer silylierten Pyrimidinbase der Formel:



worin bedeuten:

X Trialkylsilyloxy oder Trialkylsilylamino;

Y Wasserstoff, Methyl, Chlor, Fluor, Iod, Brom, Alkyl, Alkenyl, Alkynyl, Hydroxyalkyl, Carboxyalkyl, Thioalkyl, Selenoalkyl, Phenyl, Cycloalkyl, Cycloalkenyl, Thioaryl oder Selenoaryl, und

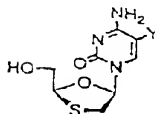
Z eine dreifachsubstituierte Silylgruppe der Formel:



worin R1, R2 und R3 unabhängig voneinander für Alkyl mit 5 Kohlenstoffatomen oder weniger oder Phenyl stehen. In Gegenwart von SnCl4.

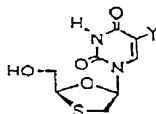
2. Verfahren nach Anspruch 1, wobei die Sauerstoffschutzgruppe [R] aus Alkyl, Silyl und Acyl ausgewählt ist.

3. Verfahren nach Anspruch 1, wobei dem Nucleosid die Formel:



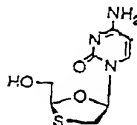
worin Y aus Wasserstoff, Halogen, Alkyl, Alkenyl, Alkynyl, Hydroxyalkyl, Carboxyalkyl, Thioalkyl, Selenoalkyl, Phenyl, Cycloalkyl, Cycloalkenyl, Thioaryl und Selenoaryl ausgewählt ist, zukommt.

4. Verfahren nach Anspruch 1, wobei dem Nucleosid die Formel:



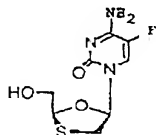
worin Y aus Wasserstoff, Halogen, Alkyl, Alkenyl, Alkynyl, Hydroxyalkyl, Carboxyalkyl, Thioalkyl, Selenoalkyl, Phenyl, Cycloalkyl, Cycloalkenyl, Thioaryl und Selenoaryl ausgewählt ist, zukommt.

5. Verfahren nach Anspruch 1, wobei dem Nucleosid die Formel:



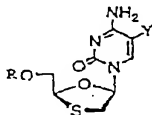
zukommt.

6. Verfahren nach Anspruch 1, wobei dem Nucleosid die Formel:



zukommt.

7. Verfahren nach Anspruch 1, bei welchem ferner zur Bildung eines 5-Oxo-1,3-oxathiolans ein Glykalddehyd der Formel OHCCH_2OR , worin R für eine Sauerstoffschutzgruppe steht, mit Thioiglykolsäure umgesetzt wird.
8. Verfahren nach Anspruch 8, bei welchem ferner
- (i) die Carbonylgruppe in dem 5-Oxo-1,3-oxathiolan zu einem Alkohol reduziert und
- (ii) der Alkohol acyliert werden.
9. Verfahren nach Anspruch 8, wobei das Reduktionsmittel aus der Gruppe Diisobutylaluminiumhydrid, Natriumbis(2-methoxyethoxy)aluminiumhydrid und NaBH_4 ausgewählt wird.
10. Verfahren nach Anspruch 1, bei welchem ferner R durch Wasserstoff ersetzt wird.
11. Verfahren nach Anspruch 10, wobei R aus einer Acylgruppe besteht und die Acylgruppe mit Natriummethoxid entfernt wird.
12. Enantiomer angereichertes 2-Hydroxymethyl-5-oxo-1,3-oxathiolan.
13. Enantiomer angereichertes 2-Acyloxymethyl-5-acyloxy-1,3-oxathiolan.
14. Enantiomer angereichertes 2-Acetoxyethyl-5-acetoxy-1,3-oxathiolan.
15. Nucleosid der Formel:

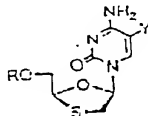


worin bedeuten:

R Wasserstoff, Alkyl, Silyl oder Acyl, und

Y Chlor, Brom, Fluor oder Iod.

16. Enantiomer angereichertes Nucleosid der Formel:

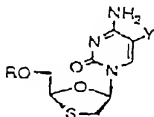


worin bedeuten:

R Wasserstoff, Alkyl, Silyl oder Acyl, und

Y Wasserstoff oder Fluor;

17. Enantiomer angereichertes Nucleosid der Formel:



worin bedeuten:

R Alkyl, Silyl oder Acyl, und

Y Chlor, Brom, Fluor oder Iod.

18. Verfahren zur Herstellung eines 1,3-Oxathiolans der Formel:

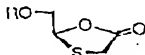


worin bedeuten:

R eine Sauerstoffschutzgruppe, und

R' eine Acylgruppe, in folgenden Stufen:

- (a) Otronisieren von $\text{CH}_2\text{CHCH}_2\text{OR}$ mit R gleich einer Schutzgruppe zur Bildung eines Glykalkaldehyds der Formel OHCCH_2OR ;
- (b) Addieren von Thio glykolsäure an den Glykalkaldehyd zur Bildung eines Lactons der Formel:



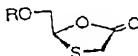
und

(c) Reduzieren des Lactons zur Bildung des 1,3-Oxathiolans.

19. Verfahren nach Anspruch 18, wobei die Reduktion des Lactons durch Zusatz eines Reduktionsmittels und anschließenden Zusatz eines Carbonsäureanhydrids bewerkstelligt wird.
20. Verfahren nach Anspruch 18, wobei das Reduktionsmittel aus der Gruppe Diisobutylaluminiumhydrid, Natriumbis(2-methoxyethoxy)aluminiumhydrid und NaBH_4 ausgewählt ist.
21. Verfahren nach Anspruch 18, wobei die Schutzgruppe aus Alkyl, Silyl und Acyl ausgewählt ist.
22. Verfahren zur Herstellung von enantiomer angereichertem 2-Acyloxy-methyl-5-acyloxy-1,3-oxathiolan in folgenden

Stufen:

(a) Vermischen eines stereoselektiven Enzyms mit einem Lacton der Formel:



worin R für eine Sauerstoffschutzgruppe steht, zur Bildung von enantiomer angereichertem 2-Hydroxymethyl-5-oxo-1,3-oxathiolan, und

(b) Reduzieren des enantiomer angereicherten 2-Hydroxymethyl-5-oxo-1,3-oxathiolans zur Bildung von enantiomer angereichertem 2-Acyloxymethyl-5-acyloxy-1,3-oxathiolan.

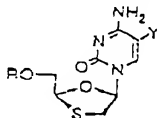
23. Verfahren nach Anspruch 22, wobei die Reduktion des enantiomer angereicherten 2-Hydroxymethyl-5-oxo-1,3-oxathiolans durch Zusatz eines Reduktionsmittels und anschließenden Zusatz eines Carbonsäureanhydrids bewerkstelligt wird.

24. Verfahren nach Anspruch 22, wobei das Reduktionsmittel aus der Gruppe Diisobutylaluminiumhydrid, Natriumbis(2-methoxyethoxy)aluminiumhydrid und NaBH_4 ausgewählt ist.

25. Verfahren nach Anspruch 22, wobei das stereoselektive Enzym aus der Gruppe Schweineleberesterase, Schweinepankreaslipase und Subtilisin ausgewählt ist.

26. Verfahren nach Anspruch 22, wobei die Schutzgruppe aus Alkyl, Silyl und Acyl ausgewählt ist.

27. Verfahren zum Auflösen der Enantiomeren eines Nucleosids der Struktur:



worin bedeuten:

Y Wasserstoff oder Fluor und

R eine Acylgruppe durch Behandeln des Nucleosids mit einem aus der Gruppe Schweineleberesterase, Schweinepankreaslipase und Subtilisin ausgewählten Enzym.

28. Verfahren nach Anspruch 27, wobei Y für Wasserstoff steht.

29. Verfahren nach Anspruch 27, wobei Y für Fluor steht.

30. Verfahren nach Anspruch 28 oder 29, wobei das Enzym aus Schweineleberesterase besteht.

31. Verfahren nach Anspruch 28 oder 29, wobei das Enzym aus Schweinepankreaslipase besteht.

32. Enantiomer angereichertes β -BCH-189.

33. Nach dem Verfahren gemäß Anspruch 28 erhaltliche Verbindung.

34. Nach dem Verfahren gemäß Anspruch 29 erhaltliche Verbindung.

35. Verbindung nach Anspruch 33 in Form des (-)-Enantiomers.

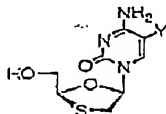
36. Verbindung nach Anspruch 33 in Form des (+)-Enantiomers.

37. β -BCH-189, im wesentlichen in Form eines einzigen optischen Isomers (vorliegend).

38. β -BCH-189 nach Anspruch 37, wobei das Isomer aus dem (+)-Enantiomer besteht.

39. β -BCH-189 nach Anspruch 37, wobei das Isomer aus dem (-)-Enantiomer besteht.

40. Verbindung der Formel

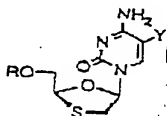


worin Y für Fluor steht.

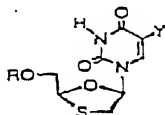
41. Das enantiomer angereicherte 5-F-Cytosinanalogue von BCH-189.

Revendications

1. Procédé de préparation d'une composition de nucléosides comprenant principalement l'isomère β du nucléoside 2'-3'-didésoxy-5'-thiazpyrimidine ayant la formule :



ou



dans laquelle R est choisi parmi le groupe constitué de l'hydrogène, d'un alkyle, du silyle et d'un acyle ; et

dans laquelle Y est choisi parmi le groupe constitué de l'hydrogène, du méthyle, du chlorure, du fluor, de l'iode, du brome, d'un alkyle, d'un alcényle, d'un alcynyle, d'un hydroxyalkyle, d'un carboxyalkyle, d'un thioalkyle, d'un sélénioalkyle, du phényle, d'un cycloalkyle, d'un cycloalcényle, d'un thioaryle et d'un sélénioaryle, comprenant l'étape de couplage d'un 1,3-oxathiane ayant la structure :



dans laquelle R' est un groupe acyle et R est un groupe protecteur de l'oxygène, avec une base pyrimidique silylée, en présence de SnCl_4 , dans laquelle la base pyrimidique silylée a la formule :

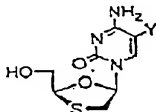


dans laquelle X est choisi parmi le groupe constitué d'un trialkylethoxy et d'un trialkylsilylamino, dans laquelle Y est choisi parmi le groupe constitué de l'hydrogène, du méthyle, du chlorure, du fluor, de l'iode, du brome, d'un alkyle, d'un alcényle, d'un alcynyle, d'un hydroxyalkyle, d'un carboxyalkyle, d'un thioalkyle, d'un sélénioalkyle, du phényle, d'un cycloalkyle, d'un cycloalcényle, d'un thioaryle et d'un sélénioaryle, dans laquelle Z est un groupe silyle tri-substitué ayant la formule :



dans laquelle R_1 , R_2 et R_3 sont choisis indépendamment parmi un alkyle possédant 5 atomes de carbone ou moins et le phényle.

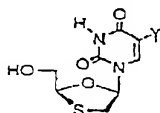
2. Procédé de la revendication 1, dans lequel [R] le groupe protecteur de l'oxygène, est choisi parmi le groupe constitué d'un alkyle, d'un alyle et d'un acyle.
3. Procédé de la revendication 1, dans lequel le nucléoside a la formule :



dans laquelle Y est choisi parmi le groupe constitué de l'hydrogène, d'un halogène, d'un alkyle, d'un alcé-

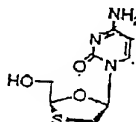
nyle, d'un alcoyle, d'un hydroxyalkyle, d'un carboxyalkyle, d'un thioalkyle, d'un sélénioalkyle, du phényle, d'un cycloalkyle, d'un cycloalcényle, d'un thiaryle et d'un sélénioaryle.

4. Procédé de la revendication 1, dans lequel le nucléoside a la formule :

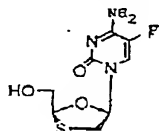


dans laquelle Y est choisi parmi le groupe constitué de l'hydrogène, d'un halogène, d'un alkyle, d'un alcényle, d'un alcynyle, d'un hydroxyalkyle, d'un carboxyalkyle, d'un thioalkyle, d'un sélénioalkyle, du phényle, d'un cycloalkyle, d'un cycloalcényle, d'un thiaryle et d'un sélénioaryle.

5. Procédé de la revendication 1, dans lequel le nucléoside a la formule :



6. Procédé de la revendication 1, dans lequel le nucléoside a la formule :



7. Procédé de la revendication 1, comprenant en outre l'étape consistant à faire réagir un glucaldéhyde ayant la formule OHCH_2OR , dans laquelle R est un groupe protecteur de l'oxygène avec l'acide thiolglycolique pour produire un 5-oxo-1,3-oxathiolane.

8. Procédé de la revendication 7, comprenant en outre :

- (i) la réduction du groupe carbonyle dans le 5-oxo-1,3-oxathiolane en un alcool ; et
- (ii) l'acylation de l'alcool.

9. Procédé de la revendication 8, dans lequel l'agent réducteur est choisi parmi le groupe constitué de l'hydruure de diisobutylaluminium, de l'hydruure de sodium bis(2-méthoxyéthoxy)aluminium et de NaBH_4 .

10. Procédé de la revendication 1 comprenant en outre le remplacement de R par de l'hydrogène.

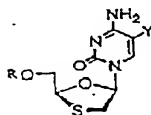
11. Procédé de la revendication 10, dans lequel R est un groupe acyle et dans lequel le groupe acyle est éliminé avec du méthylate de sodium.

12. 2-hydroxyméthyl-5-oxo-1,3-oxathiolane énantiomériquement enrichi.

13. 2-acyloxyméthyl-5-acyloxy-1,3-oxathiolane énantiomériquement enrichi.

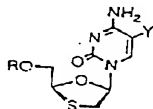
14. 2-acétoxyméthyl-5-acétoxy-1,3-oxathiolane énantiomériquement enrichi.

15. Nucleoside ayant la formule :



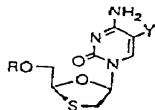
dans laquelle R est choisi parmi le groupe constitué de l'hydrogène, d'un alkyle, du silyle et d'un acyle ; et dans laquelle Y est choisi parmi le groupe constitué du chlore, du brome, du fluor et de l'iode.

16. Nucleoside énantiomériquement enrichi ayant la formule :



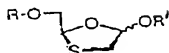
dans laquelle R est choisi parmi le groupe constitué de l'hydrogène, d'un alkyle, du silyle et d'un acyle ; et dans laquelle Y est choisi parmi le groupe constitué de l'hydrogène et du fluor.

17. Nucleoside énantiomériquement enrichi ayant la formule :



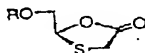
dans laquelle R est choisi parmi le groupe constitué d'un alkyle, du silyle et d'un acyle ; et dans laquelle Y est choisi parmi le groupe constitué du chlore, du brome, du fluor et de l'iode.

18. Procédé de préparation d'un 1,3-oxathiolane ayant la formule :



dans laquelle R est un groupe protecteur de l'oxygène ; et
dans laquelle R' est un groupe acyle, comprenant les étapes :

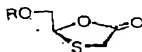
- (a) d'ozonisation de $\text{CH}_2\text{CHCl}_2\text{OR}$ pour former un glucoaldéhyde ayant la formule OHCCH_2OR , dans laquelle R est un groupe protecteur ;
(b) d'addition d'acide thioglycolique au glucoaldéhyde pour former une lactone ayant la formule :



et
(c) de réduction de la lactone pour former le 1,3-oxathiolane.

19. Procédé de la revendication 18, dans lequel la réduction de la lactone est accomplie par une addition d'agent réducteur suivie de l'addition d'un anhydride carboxylique.
20. Procédé de la revendication 18, dans lequel ledit agent réducteur est choisi parmi le groupe constitué de l'hydruure de diisobutylaluminium, de l'hydruure de sodium bis(2-méthoxyéthoxy)aluminium et de NaBH_4 .
21. Procédé de la revendication 18, dans lequel ledit groupe protecteur est choisi parmi le groupe constitué d'un alkyle, du silyle et d'un acyle.
22. Procédé de préparation de la 2-acyloxyméthyl-5-acyoxy-1,3-oxathiolane énantiomériquement enrichi, comprenant les étapes de :

(a) mélange d'une enzyme stéréosélective avec une lactone de formule :



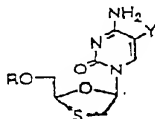
dans laquelle R est un groupe protecteur de l'oxygène, pour former le 2-hydroxyméthyl-5-oxo-1,3-oxathiolane énantiomériquement enrichi ; et

(b) réduction dudit 2-hydroxyméthyl-5-oxo-1,3-oxathiolane énantiomériquement enrichi pour former le 2-acyloxyméthyl-5-acyoxy-1,3-oxathiolane énantiomériquement enrichi.

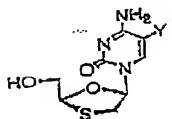
23. Procédé de la revendication 22, dans lequel la réduction du 2-hydroxyméthyl-5-oxo-1,3-oxathiolane énantiomériquement enrichi est accomplie par une addition d'un agent réducteur suivie de l'addition d'un anhydride carboxylique.

que.

24. Procédé de la revendication 22, dans lequel ledit agent réducteur est choisi parmi le groupe constitué de l'hydru-
 re de diisobutylaluminium, de l'hydru-
 re de sodium bis(2-méthoxyéthoxy)aluminium et de NaBH_4 .
25. Procédé de la revendication 22, dans lequel ladite enzyme stéréosélective est choisie parmi le groupe constitué de
 l'estérase de foie de porc, de la lipase pancréatique porcine et de la subtilisine.
26. Procédé de la revendication 22, dans lequel ledit groupe protecteur est choisi parmi le groupe constitué d'un alkyle,
 du silyle et d'un acyle.
27. Procédé de séparation des énantiomères d'un nucléoside ayant la structure :



28. Procédé de la revendication 27, dans lequel Y est l'hydrogène.
29. Procédé de la revendication 27, dans lequel Y est le fluor.
30. Procédé de la revendication 28 ou 29, dans lequel l'enzyme est l'estérase de foie de porc.
31. Procédé de la revendication 28 ou 29, dans lequel l'enzyme est la lipase pancréatique porcine.
32. β -BCH-189 énantiomériquement enrichi.
33. Composé susceptible d'être obtenu par le procédé de la revendication 28.
34. Composé susceptible d'être obtenu par le procédé de la revendication 29.
35. Composé de la revendication 33 qui est l'énantiomère (-).
36. Composé de la revendication 33 qui est l'énantiomère (+).
37. β -BCH-189 essentiellement sous la forme d'un isomère optique unique.
38. β -BCH-189 tel que dans la revendication 37, dans lequel l'isomère est l'énantiomère (+).
39. β -BCH-189 tel que dans la revendication 37, dans lequel l'isomère est l'énantiomère (-).
40. Composé de formule :



dans laquelle Y est le fluor.

41. 5-F-cytosine énantiomériquement enrichie analogue de BCH-189.

Figure 1

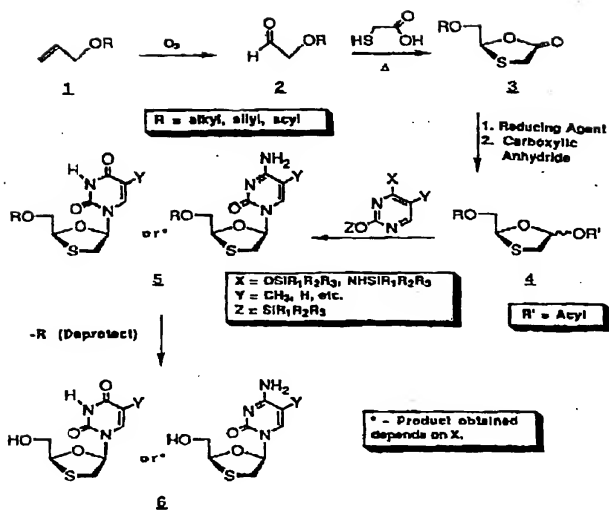


Figure 2

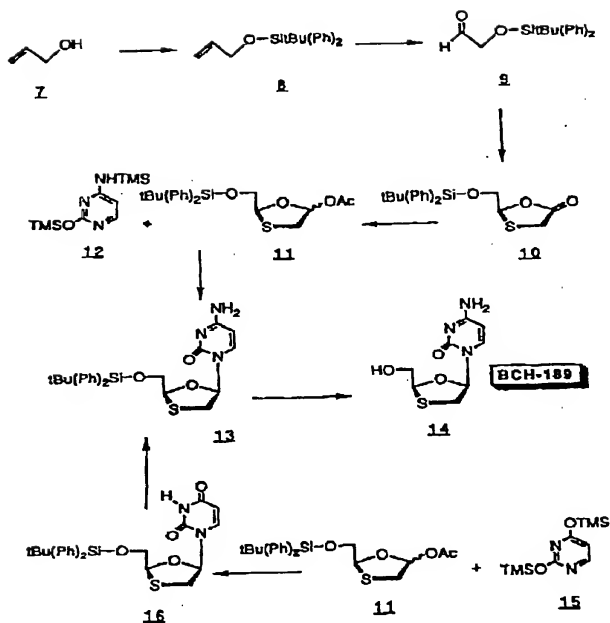


Figure 3

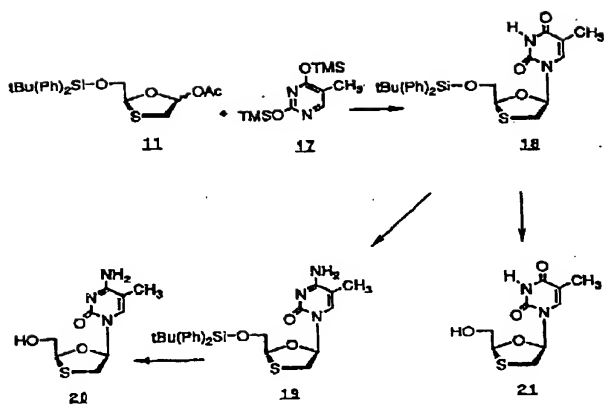
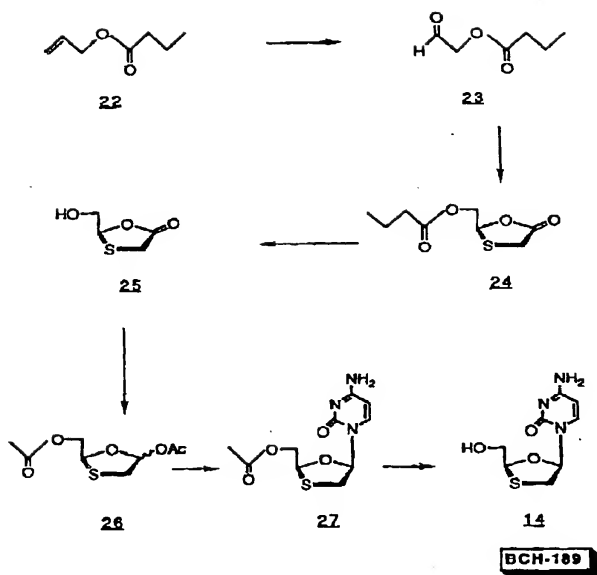
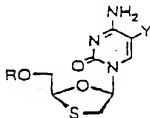


Figure 4

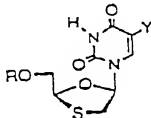


~~Amended~~ Claims

- X 1. ~~(Amended)~~. A method of preparing a nucleoside composition comprising predominantly the β -isomer of the nucleoside 2',3'-dideoxy-3'-thiapyrimidine having the formula:



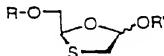
or



wherein R is selected from the group consisting of hydrogen, alkyl, silyl, and acyl; and

wherein Y is selected from the group consisting of hydrogen, methyl, chloro, fluoro, iodo, bromo, alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl,

comprising the step of coupling a 1,3-oxathiolane of the structure:



wherein R' is an acyl group and R is an oxygen-protecting group,

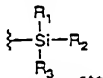
with a silylated pyrimidine base in the presence of SnCl_4 , wherein the silylated pyrimidine base has the formula:



wherein X is selected from the group consisting of tri-^{alkyl}substituted silyloxy and tri-^{alkyl}substituted silylamino;

wherein Y is selected from the group consisting of hydrogen, methyl, chloro, fluoro, iodo, bromo, alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl;

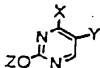
wherein Z is a tri-substituted silyl group having the formula:



wherein R_1 , R_2 , and R_3 ^{are} independently selected from alkyl possessing 5 carbon atoms or less and phenyl.

2. ~~(Amended)~~ The method of claim 1, wherein [R], the oxygen-protecting group, is selected from the group consisting of alkyl, silyl, and acyl.

~~3. The method of claim 1, wherein the silylated pyrimidine base has the formula:~~



wherein X is selected from the group consisting of trialkylsilyloxy and trialkylsilylamino;

wherein Y is selected from the group consisting of hydrogen, methyl, chloro, fluoro, iodo, bromo, alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl; and

3. The method of Claim 1, wherein the substituted pyrimidine base has the formula:

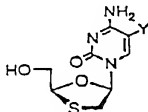


wherein X is selected from the group consisting of trialkylsilyloxy and trialkylsilylamino;

wherein Y is selected from the group consisting of hydrogen, methyl, chloro, fluoro, iodo, bromo, alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl; and

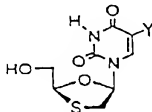
wherein Z is a trialkylsilyl group.

3. The method of Claim 1, wherein the nucleoside has the formula:



wherein Y is selected from the group consisting of hydrogen, halo, alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl.

4. The method of Claim 1, wherein the nucleoside has the formula:



3. The method of Claim 1, wherein ~~the silylated~~ pyrimidine base has the formula:

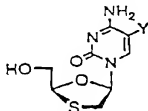


wherein X is selected from the group consisting of trialkylsilyloxy and trialkylsilylamino;

wherein Y is selected from the group consisting of hydrogen, methyl, chloro, fluoro, iodo, bromo, alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl; and

~~wherein Z~~ Z is a trialkylsilyl group.

3. The method of Claim 1, wherein the nucleoside has the formula:



wherein Y is selected from the group consisting of hydrogen, halo, alkyl, alkenyl, alkynyl, hydroxyalkyl, carboxyalkyl, thioalkyl, selenoalkyl, phenyl, cycloalkyl, cycloalkenyl, thioaryl, and selenoaryl.

4. The method of Claim 1, wherein the nucleoside has the formula:

